Comparison of Infrared Spectrophotometric and Lead Salt-Alcohol Methods for Determination of Trans Octadecenoic Acids and Esters[1]

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Lead Salt-Alcohol Method. Scope and Limitations

IN the development of analytical procedures for the separation and quantitative determination of solid fatty acids in mixtures, based on the preferential insolubility of certain fatty acid salts, a wide variety of metal salts have been investigated as precipitating agents. The other variables involved (precipitation time and temperature, solvents, etc.) have also been fairly thoroughly studied. In spite of the claims made for other methods, the lead salt-alcohol or modified Twitchell method (11, 19), which is the official method of the American Oil Chemists' Society, is probably as good as any other chemical method proposed so far.

Originally the Twitchell method was intended primarily for the separation and determination of saturated acids even though it was recognized that if myristic or other saturated fatty acids of shorter chain length were present, the separation would not be complete because the lead salts of these acids (and in some cases lead palmitate) would not precipitate quantitatively. Furthermore in analyzing tallow and hydrogenated fat, Twitchell (19) had observed that lead salts of solid unsaturated acids also precipitated with the lead salts of saturated acids. It was necessary therefore to determine the iodine number of the solid fatty acids and then calculate the percentage of solid unsaturated acids in the original sample by a simple formula (11).

In studies on the preparation of purified oleic acid from animal fats, in which selective hydrogenation was substituted for low temperature crystallization (16, 18), the lead salt-alcohol method was employed to determine the content of solid unsaturated acids (isooleic) in selectively hydrogenated tallow fatty acids and in the various fatty acid fractions obtained. Only rarely was it possible to obtain a weight balance on solid unsaturated acids. In some cases the solid unsaturated acid content calculated from analysis of the individual fractions was less than that in the starting materials, but in others it was considerably greater.

In an attempt to resolve this difficulty the lead salt-alcohol procedure was applied to a variety of pure compounds and mixtures of known composition. Table I summarizes the results obtained. This study revealed several points of interest: a) Pure oleic acid or mixtures rich in oleic acid yielded a precipitate containing large and variable amounts of lead oleate (5, 15). As a rule, sample weights much above 3.5 grams could not be used with such materials because a positive test for lead was frequently not obtained on the first filtrate. If the sample weight was less than 3.5 grams, the lead oleate usually (but not always) remained completely in solution on recrystallization. b) Determination of pure elaidic acid or mixed isooleic acids gave consistently low results. c) Application of the method to pure linoleic acid gave a waxy precipitate, which liquefied on the filter paper at room temperature. No precipitate was obtained on recrystallization. d) Stearic acid could be accurately determined either alone or when mixed with oleic acid. e) Petroselinic acid, m.p. 30.5°, behaved like a trans or lower saturated acid.

TABLE I

Analysis of Pure Fatty Acids and Mixtures of Known Composition by the Lead Salt-Alcohol Method (11, 19)

Material analyzed	Weight of sample, grams	Acid isolated from insoluble lead salt, %
Stearic acid	1.2	97-99
Oleic acid	1.0-4.0	0-35
Petroselinic acid	1.5	75-80
Elaidic acid	1.2	90-95
Linoleic acid[1]	5.0	0
Oleic-linoleic acids (80:20)	5.0	variable
Oleic-stearic acids (50:50)	2.0	48-50
Mixed isooleic acids,[2] M.P. 38°, I No. 84	1.2	85-90

[1] Prepared from tobacco seed oil fatty acids by low temperature crystallization (13).

[2] Prepared from selectively hydrogenated tallow by recrystallization of fraction P-2 and similar isooleic acid-rich fractions (18) from acetone at −20°.

The data in Table I make the results on selectively hydrogenated tallow fatty acids just described easy to understand. When fractions rich in oleic acid were analyzed for isooleic acid by the lead salt-alcohol method, the apparent total isooleic acid content of all the fractions was higher than that of the starting material. When fractions low in oleic acid but containing mainly saturated and isooleic acids were analyzed, the apparent total isooleic acid content of the fractions was lower than that of the starting material. Reasonably accurate weight balances were obtained only when the errors cancelled.

Most workers have tacitly assumed that the solid unsaturated acids isolated by the lead salt-alcohol method have the trans configuration although it is obvious from the data in Table I and other published data (5, 15) that this assumption is not valid. The two most important factors in determining whether the lead salt of an unsaturated acid will precipitate under the conditions of the method are the melting point of the acid and its content in the original mixture. Thus oleic acid, m.p. 13°, will almost invariably be isolated with the solid acid fraction when it exceeds 80% of the total acids in the mixture. Petroselinic acid, m.p. 30°, and erucic acid, m.p. 33.5°, two other cis acids, will also interfere.

In studying elaidinization equilibrium mixtures obtained from pure acids, such as oleic or petroselinic acid, in which the trans acid content is about 66%, it has been assumed that the solid unsaturated acids isolated by the lead salt-alcohol method consist solely

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of trans acids. During the earlier stages of the elaidinization reaction however, before equilibrium has been reached, the content of trans acid may still be so low that errors will be introduced by precipitation of cis acids. When the content of solid unsaturated acids is 10% or less, little, if any, will probably be isolated (9).

In addition to the specific disadvantages just described the lead salt-alcohol method is time-consuming, it requires large amounts of sample, and it does not permit much variation in the size of the sample.

Infrared Spectrophotometric Method

Observations in our laboratories (14), as well as in others (3, 4, 12), have shown that trans monounsaturated compounds have a strong, characteristic absorption in the infrared region at 10.36 microns whereas cis and saturated compounds do not. Based on this difference in absorption at 10.36 microns, an infrared spectrophotometric method has been developed by the authors (14) for the determination of trans octadecenoic acids and esters in the presence of cis octadecenoic and saturated acids and esters. The method was developed from studies on pure oleic, elaidic, petroselinic, petroselaidic, vaccenic, palmitic, and stearic acids, methyl oleate, elaidate, petroselaidate, and stearate, triolein, trielaidin, and trimyristin.

The infrared method is much more rapid, specific, and accurate than the lead salt-alcohol method, it is directly applicable to the determination of trans isomers in acid or ester mixtures; it requires only small weights of sample and the sample can be recovered.

Experimental

Materials Used. Oleic acid, b.p. 205° at 3 mm., n_D^{30} 1.4564, iodine number 90.0-90.3 (calcd. 89.9) and containing less than 0.2% of polyunsaturated acids (7), was prepared from olive oil fatty acids by distillation and low temperature crystallization (8, 17). Elaidic acid, m.p. 44° and iodine number 89.5, was prepared by isomerization of oleic acid with 0.3% of powdered selenium at 225° for one hour, followed by fractional crystallization of the reaction products from acetone twice at —20° and three times at 0 to 5° (5 ml. of solvent per gram of solute). Petroselinic acid (cis-6-octadecenoic acid), m.p. 30.5-31.0° and iodine number 88.8, was prepared from celery seed oil by methanolysis, fractional distillation to isolate the C-18 fraction, conversion to free acids, and five recrystallizations from acetone at —20° and one at 0° (10 ml. of solvent per gram of solute). Petroselaidic acid (trans-6-octadecenoic acid), m.p. 52.8-53.2° and iodine number 89.8, was prepared by isomerization of petroselinic acid with powdered selenium and recrystallization of the reaction mixture three times from acetone at 0° (10 ml. of solvent per gram of solute). Palmitic acid, m.p. 62.1-62.5° and neutralization equivalent 256.3 (calcd. 256.4), was prepared by recrystallization of the 90% commercial grade from acetone at 0° (10 ml. of solvent per gram of solute). Stearic acid, m.p. 69.4-69.6° and neutralization equivalent 282.4 (calcd. 284.5), was prepared by recrystallization of the fatty acids from completely hydrogenated soybean oil from acetone at 0° (10 ml. of solvent per gram of solute). Methyl oleate, b.p. 155° at 0.15 mm. and n_D^{30} 1.4484, methyl elaidate, b.p. 179-180.4° at 4 mm. and n_D^{30} 1.4492, methyl petroselaidate, iodine number 85, and methyl stearate, b.p. 180° at 4 mm., m.p. 38-39° and n_D^{40} 1.4363, were prepared by esterification of oleic, elaidic, petroselaidic, and stearic acids respectively, with anhydrous methanol containing small quantities of sulfuric acid as catalyst, followed by fractional distillation of the neutralized reaction mixture. Trielaidin, m.p. 41.3-41.5°, trimyristin, m.p. 58°, and triolein, iodine number 85.1, were prepared by the reaction of a benzene solution of the corresponding acid chlorides (2) with a pyridine solution of glycerol, followed by recrystallization from appropriate solvents. Tristearopalmitin was obtained by recrystallization of completely hydrogenated vegetable oil from acetone.

Lead Salt-Alcohol Method. Official method Cd 6-38 of the American Oil Chemists' Society was used (11).

Determination of Extinction Coefficients (k). A Beckman IR-2 infrared spectrophotometer was employed. All measurements were made in a liquid cell, which consisted of two rock salt windows separated by an amalgamated lead spacer. The cell thickness was 0.1054 cm.

Approximately 0.25 gram of each pure compound was accurately weighed into a 25-ml. volumetric flask and diluted to the mark with pure carbon bisulfide. The solution was allowed to stand in the tightly stoppered flask for at least one hour in an air-conditioned room at 25 ± 0.1°. All measurements were made under these conditions. A portion of the solution was introduced into the absorption cell by a hypodermic syringe, and the optical density of solution plus cell at exactly 10.36 microns was measured by the null method. The optical density at 10.36 microns of the cell filled with carbon bisulfide was then measured under identical conditions, and the difference was taken as the absorption due to the solute. Similar measurements were made with different concentrations of solution prepared either by dilution of the original or by weighing different quantities of solute. When only small quantities of reference compounds were available, a 2-ml. volumetric flask and a semimicro balance were employed. Solutions containing from 0.001 to 0.04 mole of solute per liter were investigated. Over this range plots of optical density as a function of concentration showed excellent adherence to Beer's law. During the entire investigation the optical density of the cell filled with solvent remained constant within 0.002 optical density unit. Extinction coefficients, k, were calculated from the formula $k = d/cl$, where d is optical density, c is concentration in grams per liter, and l is cell thickness in cm. (0.1054).

Analysis of Mixtures. When possible, the concentration of the solution was selected so as to give a corrected optical density between 0.2 and 0.5, and the measurements were made exactly as described above. It was not possible to obtain solutions having optical densities in this optimum range when the content of trans component was low. Dividing the corrected optical density of the unknown solution at 10.36 microns by its concentration in grams per liter and the cell thickness yields the observed extinction coefficient ($k_{ob.}$) to be used in the calculation of percentage of trans components.

Results

Table II gives the extinction coefficients employed in the infrared spectrophotometric method for determination of trans octadecenoic acids and esters.

Calculations. In unknown acid mixtures the k_{trans} value employed is the average of the k values reported for the trans acids in Table II. If the mixture contains both saturated and cis monounsaturated acids, the k values for palmitic, stearic, and the cis octa-

TABLE II

Extinction Coefficients at 10.36 Microns for Pure Cis and Trans Monounsaturated and Saturated Acids and Esters (14)[1]

Compound	Extinction coefficients, k[2]
Oleic acid	0.133
Petroselinic acid	0.129
Palmitic acid	0.129
Stearic acid	0.123
Elaidic acid	0.552
Petroselaidic acid	0.560
Methyl oleate	0.041
Methyl stearate	0.028
Methyl elaidate	0.442
Methyl petroselaidate	0.456
Triolein	0.084
Trimyristin	0.087
Tristearopalmitin	0.078
Trielaidin	0.475

[1] Cell thickness 0.1054 cm.; slit width, 0.39 mm.

[2] $k = \frac{\text{optical density at 10.36 microns}}{\text{(concentration in g./liter) (cell thickness in cm.)}}$

decenoic acids are averaged ($k_{av.}$), and this value is used in the calculation given below. For acid mixtures known to contain little or no saturated acids, the average of the k values for oleic and petroselinic acids is used (k_{cis}). Likewise for acid mixtures containing little or no unsaturated acids, the average of the k values for palmitic and stearic acids is used for $k_{av.}$ in the calculation. Similar considerations apply for the esters and glycerides.

a) Mixtures presumably containing cis and trans octadecenoic components only, such as elaidinization mixtures:

$$\text{Trans component, \%} = \frac{100\,(k_{ob.} - k_{cis})}{k_{trans} - k_{cis}}$$

b) Mixtures containing cis and trans octadecenoic and saturated components:

$$\text{Trans component, \%} = \frac{100\,(k_{ob.} - k_{av.})}{k_{trans} - k_{av.}}$$

Table III gives the percentage of trans component obtained by the infrared method on several known synthetic mixtures of pure compounds as well as the percentage of solid unsaturated acids by the lead salt-alcohol method (11). Table IV gives similar information on mixtures of unknown composition.

Discussion

The results in Table III show that the infrared method has a high degree of precision and accuracy, all mixtures giving acceptable analyses.

Except when the oleic acid content of the mixture is high, the lead salt-alcohol method consistently gives low results (Tables III and IV), and the error is often considerable. The data for the lead salt-alcohol method in Table III show an interesting trend. Of the four results, one was substantially correct, one was more than 1000% high, and the remaining two were low, the magnitude of the negative error increasing as the content of trans component decreased. The single correct result was purely fortuitous and was obtained only because the failure to isolate elaidic acid quantitatively (see Table I) was compensated for by isolation of the necessary amount of oleic acid. Infrared analysis demonstrated conclusively that the acids isolated did not consist exclusively of the trans isomer but contained only about 85-90%. The extremely high value was caused by precipitation of large amounts of lead oleate with lead elaidate. This was confirmed by the fact that the acids isolated were liquid, and infrared analysis indicated the presence of only about 10% of trans isomer. The low values represent the normal behavior of the lead salt-alcohol method in isolating trans acids, and it was expected that the error would increase as the content of elaidic acid decreased (9).

The data in Table IV emphasize even further the great disparity between the lead salt-alcohol and the infrared methods and present several interesting highlights. Although the infrared method indicates that the equilibrium composition of elaidinization reactions is approximately two-thirds trans component, and this is in agreement with the literature (6, 20), the values obtained by the lead salt-alcohol method decrease with increasing reaction time beyond one hour. We have explained this on the assumption that small amounts of polymeric acids form during long-time heating with selenium at 220-225°, and these exert a solubilizing effect on the lead salts of the trans acids formed. Furthermore saturation of double bonds is probably occurring during reaction with selenium, as shown by the fact that the solid acids isolated by the lead salt-alcohol procedure have iodine numbers from 2-7 units lower than the calculated values. The elaidinization reaction is apparently more complex than has usually been assumed, and perhaps it also involves double bond shifts, but this does not appear to affect the accuracy of the infrared method.

The purified oleic acid, iodine number 91.5, prepared from selectively hydrogenated tallow (16, 18), contains about 25% of trans octadecenoic acids; the oleic acid, iodine number 78.3, prepared from red oil by dimerization (10) of the linoleic acid fraction, contains about 40-45%, and the oleic acid, iodine number 88.2, prepared from red oil by a combination of fractional distillation and low temperature crystallization (17), contains less than 4% of trans acids.

The four materials listed consecutively in Table IV, starting from purified oleic acid, iodine number 91.5, and including fatty acids, iodine number 41.7, are the three fractions isolated and the starting material in the preparation of a purified oleic acid from selectively hydrogenated tallow (16, 18). The three fractions amount to 33, 44, and 10% respectively, of the

TABLE III

Analysis of Synthetic Mixtures of Pure Acids and Esters

Composition of Mixture[1]			Trans component determined by infrared method, %			Error, %	"Isooleic" acids determined by lead salt-alcohol method (11), %	Error, %
ME	MO	MS	(1)	(2)	(Av.)			
66.91	33.09	0	66.52	66.81	66.67	−0.4	.....[2]	.....
18.31	81.69	0	18.78	18.46	18.62	+1.6	.....	.....
3.12	96.63	0	3.20	3.38	3.29	+5.4	.....	.....
26.12	23.87	50.01	25.47	25.68	25.53	−2.3	.....	.....
2.31	13.20	84.49	2.09	2.22	2.16	−6.5	.....	.....
EA	OA	SA						
67.48	32.52	0	67.11	67.52	67.32	−0.2	.....[2]	.....
46.90	53.10	0	45.24	45.60	45.42	−3.2	46.5	−0.9
3.31	96.69	0	3.50	3.80	3.65	+10.3	38.1[3]	+1055
21.24	25.29	53.47	22.10	22.42	22.26	+4.8	18.1	−14.5
10.32	7.07	82.61	10.51	10.73	10.63	+3.0	6.6	−35.9
2.26	20.76	76.97	1.67	2.02	1.85	−18.0	.....	.....

[1] ME = methyl elaidate; MO = methyl oleate; MS = methyl stearate; EA = elaidic acid; OA = oleic acid; SA = saturated acids, consisting of equal parts of palmitic and stearic acids.

[2] See Table IV for analysis of mixtures of approximately this composition.

[3] Acids isolated were either liquid or semi-solid.

starting material and contain a total of 82% of the trans acids originally present, as determined by infrared analysis. About 13% of the total fatty acids was lost however, mainly as distillation residue and low boiling fractions, and this material contained about 23% of the trans acids (not shown in Table IV). By a simple calculation it can be shown that the trans acid content of the various fractions totals 104% of that originally present, which is an excellent check,

TABLE IV

Analysis of Unknown Mixtures

Material analyzed	Trans component determined by infrared method, %	"Isooleic" acids determined by lead salt-alcohol method (11), %
Elaidinization mixture A[1]	67.3[2]	57.8
Elaidinization mixture B[3]	67.0[2]	42.3
Elaidinization mixture C[4]	69.7[2]	61.8
Elaidinization mixture D[5]	67.9[2]	43.5
Elaidinization mixture E[6]	65.0	55.8
Purified oleic acid, I. No. 91.5, octadecenoic acid content 95%, from selectively hydrogenated tallow (16, 18)	24.8	11.8[7]
Saturated acids, I. No. 3.0, from selectively hydrogenated tallow (16, 18)	3.3	1.3
Saturated acids, I. No. 14.8, from selectively hydrogenated tallow (16, 18)	14.9	5.6
Fatty acids, I. No. 41.7, octadecenoic acid content 45%, from selectively hydrogenated tallow (16, 18)	13.5	4.0
Fatty acids, I. No. 38.5, octadecenoic acid content 42%, from selectively hydrogenated tallow (16, 18)	15.6	5.2
Purified oleic acid, commercial (10), I. No. 78.3, octadecenoic acid content 83%	42.6	18.4[7]
Purified oleic acid, I. No. 88.2, from red oil (17), octadecenoic acid content 98%	3.8	0-35[7]
Olive oil, edible grade	0	20.5[7](15)
Hydrogenated vegetable oil, edible grade, commercial	34.2	13.1
Mixed isooleic acids,[8] I. No. 84	91.2	82.7

[1] Oleic acid heated for 1 hour at 220-5° with 0.3% powdered selenium.
[2] Literature (6) gives 66.7% determined by the iodine equilibrium method (20).
[3] Oleic acid heated for 3 hours at 220-5° with 0.3% powdered selenium.
[4] Elaidic acid heated for 1 hour at 220-5° with 0.3% powdered selenium.
[5] Elaidic acid heated for 3 hours at 220-5° with 0.3% powdered selenium.
[6] Petroselinic acid heated for 3 hours at 220-5° with 0.3% powdered selenium.
[7] Acids isolated were either liquid or semi-solid.
[8] Prepared as described in Footnote 2 of Table I.

considering the number of fractions involved and their different nature. Assuming that the original content of trans acids determined by infrared analysis is correct, the total accounted for by the lead salt-alcohol method is only about 50% of that calculated.

As determined by the infrared method, olive oil, edible grade, contains no trans isomers whereas the lead salt-alcohol method indicates a content of trans isomers of as much as 20%. The acids isolated by the lead salt-alcohol method from olive oil fatty acids are semi-solid. Furthermore by the selection of a small enough sample (less than about 3.5 grams) of olive oil fatty acids for use in the lead salt-alcohol procedure, a value of zero can be obtained for its "isooleic" acid content. On the basis of the results by the infrared method however olive oil still remains the best laboratory source of pure cis-9-octadecenoic acid.

A commercial, edible grade of hydrogenated vegetable oil was found to contain 34.2% trans glycerides by the infrared method but only about 13.1% by the lead salt-alcohol method. The highest value we have seen in recent years for the "isooleic" content of hydrogenated vegetable oils, determined by the lead salt-alcohol method, is 20.4% (1), a value probably much below that actually present.

General Comments

Because of the present state of development of infrared spectrophotometry, the extinction coefficients reported in this paper cannot be employed directly by other workers. Variations in such factors as scattered radiation, wave length calibration, slit width settings, and cell thickness, in different instruments, make it necessary for the analyst planning to use the method to determine the extinction coefficients on the instrument being used under the exact conditions employed in the analysis. It is probably unnecessary to redetermine the extinction coefficients of all the compounds reported in Table II, but a member of each class should certainly be examined. The molecular vibration corresponding to the 10.36 micron wave length probably occurs in both cis and trans olefinic compounds but is not infrared active in the normal cis fatty acids because of symmetry. We are investigating the possibility that some commonly occurring side groups in close proximity to the ethylenic system might destroy the symmetry enough to cause even cis fatty acids to show absorption at 10.36 microns. Until we have settled this question, we recommend that the infrared method of analysis for trans constituents be restricted to saturated and monounsaturated fatty acids and esters which are known to be free of branching or other substituents close to the ethylenic system.

To investigators conducting research on the oxidation, isomerization, polymerization, composition, and hydrogenation of fats and their components and derivatives, and on the preparation of pure unsaturated acids and esters, the infrared spectrophotometric method is suggested as a necessary tool.

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Summary

The scope and limitations of the lead salt-alcohol method (11, 19) have been defined as a result of observations in the authors' laboratories and in others. Trans octadecenoic acids and esters are not determined specifically by this method, and when large amounts of cis acids or esters are present, they are also isolated with the solid acid fraction.

The infrared spectrophotometric method, previously described by the authors (14), and the lead salt-alcohol method have been applied to a variety of synthetic mixtures of known composition and to other materials. Comparison of the data indicates that the infrared method is more rapid, specific, and accurate than the lead salt-method; it is directly applicable to the determination of trans isomers in acid or ester mixtures; only small samples are required; and they can be recovered if necessary.

The infrared method is suggested as a necessary tool to investigators conducting research on the oxidation, isomerization, polymerization, composition, and hydrogenation of fats and their components and derivatives, and on the preparation of pure unsaturated acids and esters.

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